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THE FAILURE OF ETHER TO PRODUCE MUTATIONS IN DROSOPHILA

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THE many mutants of *Drosophila ampelophila* that have appeared "under domestication" have raised the question as to the cause or causes that have brought about the result. Since every fly that has passed through our hands has been *etherized* once in its life, usually before it begins to lay its eggs if a female or before mating if a male, it might appear that this recurring condition was responsible for the mutations. At any rate it seemed worth while to put this view to a test, if for no other reason than to remove from one's mind the suspicion that ether "did it."

Preliminary trials showed that two drops of ether (on a piece of cotton) in a quart milk bottle, tightly stoppered with a cotton plug, would not noticeably affect the flies in half an hour, three drops made them slightly "stupid," four drops more so, and five drops quieted them. It was found that they would for the most part recover even after 6, 7 and 8 drops of ether. If etherized twice daily the flies were so far weakened that they generally died without laying any eggs. Therefore in the later experiments the flies were etherized only once a day or once in two days.

The larvæ (beginning two days after the eggs were laid, at which time the eggs have hatched) can stand more ether. Twice daily throughout their larval and pupal lives (approximately 11 days) 6, or 7, or 8, or 9 or even 10 drops of ether were added to the quart bottles. The ether excited the larvæ at first, then quieted them; later they recovered. In such tests the larvæ were kept almost continuously in an atmosphere of ether from birth to emergence of the fly and in a few cases the etherization was continued with the flies also. By covering a wide range of stages and conditions I hoped to find the critical point, if any such existed, when ether would act. Since, as the sequel will show, no specific results were obtained it seems unnecessary to give the details of all these trials.

Double, and in one case triple recessive, stocks were used for the work, because experience had already shown that even with great care contamination may occur. One or two flies that came from escaped mutants would ruin the value of the data, but the operator can protect himself by using stocks that have already two or more recessive characters. If such flies mutate in one of the characters involved the presence of the other one will make it certain that the mutant belonged to this culture, and had not come in from outside; if a change appeared in some other part, the double recessive character would still identify the stock. Two of the stocks used had sex-linked characters, *i. e.*, eosin miniature and cherry club vermilion. If a mutation should appear that involved these characters it would become evident at once in the male offspring; for, the male gets his single sex chromosome from his mother and exhibits her sex-linked factors. Of course this would be equally true for any other sex-linked character that appeared, but in practise it is impossible to thoroughly examine each fly in every possible part, so that I had to confine my attention to certain organs, and in these cases I concentrated on the mutant characters. Conspicuous mutations in other parts would, I think, have been picked up, but minor ones would probably have been missed. On the other hand, if changes taking place in the chromosomal material are the basis for mutation it would seem perhaps a priori unlikely that the same changes should occur at the same time in both members of a pair, and if not the effect would not appear in the next generation, and not until two flies of the later progeny each carrying one mutant factor met. Whatever weight may be attached to this argument—we know really nothing as to the origin of mutations—it seemed necessary to carry some stocks to another generation; and this was done.

The following are the totals of offspring produced by flies from larvæ that had been etherized twice daily from the time of hatching to the winged state:

Black vestigial	310 556
Pink black	1,390
Eosin miniature	871
Cherry club vermilion	364
Pink ebony sepia	1,311
Total	4,802

In the next case fewer drops of ether were used—four or five throughout larval and pupal life. The parent flies were changed to new bottles quite often to prevent crowding and abundant food was supplied:

Black vestigial	2,122
Pink black	6,762
Eosin miniature	2,603
Pink ebony sepia	953
Total	12,440

The following data are from the offspring of the flies that had 8 and 10 drops of ether twice daily:

Pink black	3,440
Eosin miniature	2,775
Total	6,215

The next data are the records of the offspring of adult flies that had been etherized several times (usually twice a day) just after they had hatched:

Black vestigial (6 times)	870
Black vestigial (7 times)	143
Black vestigial (8 times)	694
Pure black (6 times)	81
Eosin miniature (3 times)	206
Eosin miniature (5 times)	428
Cherry club vermillion (8 times)	713
Cherry club vermillion (8 times)	476
Total	3,611

Finally some of the flies that had appeared, in the experiments in which 8 and 10 drops of ether had been used throughout the larval and pupal life, were bred and gave in the next generation the following records:

Pink black	2,186
Eosin miniature	666
Cherry club vermillion	709
Pink ebony sepia	539
Total	4,100

In a grand total of 31,168 flies subjected to ether, there was not a single mutation observed. It seems highly probable therefore that ether has no specific effect in producing mutations in *Drosophila ampelophila*. It might, of course, still be said that mutations are so rare, that, although caused by ether, they still are not frequently enough produced to make 31,000 flies a sufficient guarantee. Granting this, it still remains that since no mutants appeared under this excessive treatment, ether does not play the rôle of a specific agent causing the mutations of *Drosophila*, and one is inclined to look elsewhere for a solution of the problem.

One of the first mutants that I observed in *ampelophila* appeared in the offspring of flies that had been treated with radium and although there was no proof that the radium had had a specific effect I felt obliged to state the actual case, refraining carefully from any statement of causal connection.[1] Nevertheless, I have been quoted as having produced the first mutants by the use of radium. I may add that repetition of the experiment on a large scale both with the emanations of an X-ray machine and from radium salts has failed to produce any mutations, although the flies were made sterile for a time. Loeb and Bancroft also tried the effect of radium.[2] They found a black mutant type after treatment with radium but since the same type appeared in the control they do not believe that its appearance had any connection with the radium. They also state that after treatment a white-eyed female appeared in the first generation, and suggest that a white eyed male may have existed in a previous generation that escaped notice, but if it had been found in a previous generation, the mutation or the contamination must have been earlier than the one that produced the white-eyed female; for, a white-eyed male takes two generations to reappear again. Pink-eyed flies were also found both in the control and in the treated flies. In regard to another mutant type, they state:

> We succeeded in producing short winged specimens in two different cultures by treating them with radium, while thus far we have not observed this mutation in cultures not treated with radium.

But although "two hundred different cultures" were subsequently treated with radium and no short-winged (miniature) flies appeared, I get the impression that Bancroft and Loeb must have had stock that was already contaminated by some recessive mutant factors. All of these mutants had been obtained and described by us, and the stock used by Bancroft and Loeb was obtained in part at least from my friend Dr. Frank E. Lutz, who had at that time in his possession, as a letter I have from him states, certainly two of these mutants, black and miniature, that he had received from me. It seems to me not improbable that the collector, who got the stock from Dr.

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[1] *Science*, XXXIII, 1911.

[2] *Loc. cit.*

Lutz for Professor Loeb, included by mistake some flies heterozygous for these two characters; for in our very extensive experience with wild stock from Cold Spring Harbor (the origin of most of Dr. Lutz's stock) and elsewhere these mutants have never arisen again.

At various times experiments have been made in this laboratory involving wide ranges of temperature,[3] salts, sugars, acids, alkalis without any resulting mutation. In fact, our experience with *Drosophila* has given us the impression that mutations are rare events, although the actual number of our mutants is now quite large.

Guyénot[4] also has treated *ampelophila* to high temperatures, to radium and to X-rays without result. When the adult flies were treated with ultra-violet light, however, a definite type of "black" fly was obtained. The first eggs that such females lay are normal and give rise to normal flies. The eggs laid later fail to hatch, although they appear to begin their development. On the third day amongst the abnormal eggs some were found that gave rise to flies that were apparently normal. It happened that they were not examined again until after the flies of the next generation had appeared (many of them had died). Both among the living and the dead flies there was a considerable percentage of black flies. The black females laid eggs which did not develop, even although normal males were added. Why the black males were not also tested by outcrossing is not apparent. The description of the black flies given by Guyénot tallies in some points with our stock of ebony in which the females were at first usually infertile but the males fertile. At first, indeed, we kept the stock by breeding the ebony males to the heterozygous females. These are intermediate in color. In fact, Guyénot seems to have had heterozygous flies but did not, according to his account, obtain any black flies from them. However, if the ultra-violet light is a specific agent for these mutations the experiment can easily be repeated.

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[3] *Science*, XXII, 1910.

[4] *Bull. Scientifique*, XLVIII, 1914.

It should be added that only one of Guyénot's two lines gave dark flies after treatment with ultra-violet light. This might seem to indicate that the first result was accidental, or due to the presence of a recessive mutation in the stock prior to treatment were it not that a careful control is recorded. Guyénot himself speaks with much caution concerning the interpretation of his results. Decision as to their value may be reserved until repetition of the experiment gives confirmation. Our own experience with *Drosophila* shows that mutations appear under conditions where all the other flies in the same culture are normal and we have become unduly sceptical perhaps towards evidence which refers a particular mutant to some unusual treatment to which the flies have been subjected. Until we can get definite information as to how mutants arise, whether through external influences, through accidents of mitosis, through hybridizing, or through changes in the chromosomes with its consequent dislocations of the machinery of crossing over, or in some other way, it seems futile to discuss the question.